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INVESTIGATIONS IN FISH CONTROL

22. Efficacy of Quinaldine as an Anesthetic for Seven Species of Fish

23. Toxicity of Quinaldine to Selected Fishes

24. Quinaldine as an Anesthetic for Brook Trout, Lake Trout, and Atlantic Salmon

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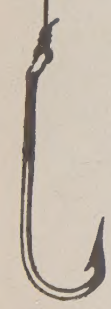

United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

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INVESTIGATIONS IN FISH CONTROL

22. Efficacy of Quinaldine as an Anesthetic for Seven Species of Fish

By Richard A. Schoettger and Arnold M. Julin, Fishery Biologists

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
Washington, D.C. · January 1969

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EFFICACY OF QUINALDINE AS AN ANESTHETIC FOR SEVEN SPECIES OF FISH

By Richard A. Schoettger and Arnold M. Julin, Fishery Biologists
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

ABSTRACT.--Quinaldine was tested as an anesthetic for rainbow trout, brown trout, brook trout, lake trout, channel catfish, bluegill, and largemouth bass. In general, 15 to 70 ppm of the drug induce total loss of equilibrium in fish within two minutes. Efficacy is influenced by acid pH and for some species, by temperature, but not by water hardness, age of quinaldine solutions, or repeated exposures of fish to quinaldine. Assets include rapid action and prolonged maintenance of anesthesia, but anesthetized fish retain a degree of reflex responsiveness which may interfere with stripping, delicate surgical operations, and blood collection. The drug is harmless to fertilized rainbow trout eggs at concentrations and exposure times normally encountered in spawning operations.

Quinaldine (2-methylquinoline) is obtained from coal tar and is used in the manufacture of dyes (Turner, 1950). Its anesthetic effect on fish was first reported by Muench (1958), who found that concentrations of 2.5 to 20 ppm narcotized goldfish, golden shiners, yellow bullheads, green sunfish, and white crappies within 0.5 to 4 minutes. The relatively small number of published reports on the use of quinaldine as a fish anesthetic, in contrast to MS-222 (Schoettger, 1967), suggests that it is not widely used by fishery workers. However, in our survey of chemicals used at national fish hatcheries, we found that it is employed in handling a variety of species including sockeye salmon, chinook salmon, coho salmon, rainbow trout, brown trout, brook trout, northern pike, goldfish, channel catfish, smallmouth bass, largemouth bass, and walleye. Natarajan and Ranganathan (1960) used quinaldine in fish transport, and Greenough (1963) patented a fish-transport medium containing a buffer, an antibiotic, and quinaldine. Penfold (1965) found the anesthetic useful in the live collection of certain marine species.

Recent amendments to the Federal Food, Drug, and Cosmetic Act require that chemicals used on fish be cleared and labeled for their specific uses (Lennon, 1967). The information needed to clear quinaldine includes its toxicity to fish, its efficacy as an anesthetic, its residues in fish tissues, and its safety to other animals. The intent of our research was to extend Muench's (1958) investigations on efficacy to include species more commonly cultured in hatcheries, such as fingerling and adult trout, catfish, and centrarchids. Also, we wished to study the influence of temperature, water quality, and repeated narcosis of fish on the efficacy and stability of quinaldine solutions, and the toxicity of quinaldine to trout eggs.

Spector (1956) cited an oral LD_{50} for rats of 1.23 g./kg. and a cutaneous toxicity to rabbits of 1.87 g./kg. Both values classify quinaldine as slightly toxic to mammals. Bell (1964) recommended avoidance of vapor inhalation and prolonged skin and eye contact. According to personal communication from Dr. Hans L. Falk, Associate Director for

Carcinogenesis, National Cancer Institute, Department of Health, Education and Welfare, Bethesda, Maryland, November 10, 1966, there is no evidence that quinaldine has carcinogenic properties, either on the basis of bioassay, or on correlation with structural requirements for carcinogenicity. Quinaldine N-oxide, the only related chemical they tested for carcinogenicity, was inactive.

METHODS AND MATERIALS

Quinaldine used in these investigations was 95 percent technical material purchased from Eastman Kodak Company. Efficacy of the chemical was tested with rainbow trout, brown trout, brook trout, lake trout, channel catfish, and largemouth bass of two to six inches and seven to 12 inches (table 1). Bluegills of two to six inches were also tested. The trials with trout were conducted at 7°, 12°, and 17° C., and those with channel catfish and centrarchids at 7°, 12°, 17°, 22°, and 27° C.

The methods of preparing test solutions and various water qualities, acclimating the test fish, and evaluating efficacy were essentially the same as those described by Schoettger and Julin (1967), with some modifications. The major modifications were in the preparation of stock solutions and in the criteria for efficacy. Stock solutions were prepared by diluting the compound with acetone and water. Sufficient acetone was added to produce clear solutions.

The changes in criteria for efficacy were related to the differences in behavioral responses of quinaldine-treated and MS-222-treated fish. The responses of the former are discussed later. Concentrations inducing total loss of equilibrium (stage 11) within 2 minutes were considered effective. Following application of anesthesia, the fish were held in the test solutions until they entered medullary collapse, or for 6 hours, whichever occurred first. This period gave a measure of the tolerated exposure time. The fish were then placed in flowing well water for recovery.

During the investigations, we observed that pH influenced the effectiveness of quinaldine. Additional trials were carried out to better define this influence and to determine whether it was reversible with changes in pH. Smallmouth bass were tested in solutions containing 20 and 30 ppm of quinaldine; channel catfish in 50 and 60 ppm. The pH of solutions was manipulated with 1N sodium hydroxide and 0.25M phthalic acid. The test solutions were maintained at 12° C. in water baths.

The influence of water hardness on the efficacy of quinaldine was measured with 2.5-inch rainbow trout at 12° C. Solutions of 10 and

Table 1.--Species and sources of fish

Common name	Scientific name	Source
Rainbow trout....	Salmo gairdneri	NFH,1/ Manchester, Iowa
Brown trout.....	Salmo trutta	SFH,2/ Lanesboro, Minn.
Brook trout.....	Salvelinus fontinalis	SFH, Lanesboro, Minn.
		SFH, Osceola, Wis.
Lake trout.....	Salvelinus namayacush	NFH, Jordan River, Charlevoix, Mich.
		SFH, St. Croix Falls, Wis.
Channel catfish..	Ictalurus punctatus	NFH, Fairport, Iowa
		NFH, Guttenberg, Iowa
		SFH, Lansing, Iowa
Bluegill.....	Lepomis macrochirus	NFH, Guttenberg, Iowa
		NFH, Lake Mills, Wis.
Smallmouth bass..	Micropterus dolomieui	NFH, Fairport, Iowa
Largemouth bass..	Micropterus salmoides	NFH, Genoa, Wis.

1/ National Fish Hatchery
2/ State Fish Hatchery

180 ppm of total hardness were prepared as described by Schoettger and Julin (1967) and contained 15 ppm of quinaldine. Ten fish were used to bioassay each test solution. Effects on efficacy were judged by the times to induce total loss of equilibrium (stage 11) in all fish.

The effect of repeated exposures to quinaldine was tested by treating ten 3.6-inch rainbow trout daily in a concentration of 12 ppm at 12° C. The times to total loss of equilibrium (stage 11) and recovery, and the percent survival were used as indexes of sensitivity. After six consecutive treatments, the susceptibility of the fish was compared with that of untreated controls.

The potency of quinaldine solutions aged up to 50 days at 12° or 27° C. were determined by bioassay. The tests at 12° were conducted in polyethylene tanks containing 45 liters of a 15-ppm solution. Temperature was maintained within ± 2° C. by water baths, and at intervals efficacy was checked against ten 5-inch rainbow trout. Two-inch bluegills were used in bioassays at 27° since trout are difficult to maintain at this temperature. A level of 12 ppm was tested at 27° because, at the time, effective concentrations had not yet been established for bluegills. The volume of some solutions at both temperatures was reduced during aging by evaporation, but oxygen levels were not seriously affected. Water losses were replaced with deionized water before the tests.

The quantity of fish which can be anesthetized per milliliter of quinaldine was estimated by narcotizing a number of 5-inch rainbow trout in the same solution until it became ineffective. The test was conducted in 2.5 liters of a 15-ppm solution which was aerated and maintained at 12° C. Five individuals at a time were anesthetized to total loss of equilibrium (stage 11) removed, weighed, and placed in fresh water. None of the fish was exposed to the chemical more than once. The test solution was considered ineffective when, in consecutive trials, fewer fish were anesthetized.

The toxicity of quinaldine to fertilized eggs of rainbow trout was determined at concentrations of 15, 30, 60, and 120 ppm. Sixty to 70 eggs, 24 hours old, were placed in 2.5 liters of each concentration. Fifteen eggs were removed from each solution after 15, 30, 60, and 120 minutes of exposure, rinsed, placed in petri dishes, and incubated in well water at 12° C. Four groups of control eggs were placed in reconstituted water without quinaldine and then incubated like the treated eggs. Mortalities among the quinaldine-treated and control eggs were recorded 96 hours after treatment, and thereafter at intervals until hatching.

RESULTS AND DISCUSSION

BEHAVIOR OF ANESTHETIZED FISH

The behavior of quinaldine-treated fish is different, in some respects, from that of fish exposed to other anesthetics (McFarland, 1960; Schoettger and Julin, 1966). At first, the chemical causes irritation which seems to increase in intensity with concentration. Shortly thereafter they lose equilibrium without entering a pronounced stage of sedation. Anesthesia progresses rapidly to total loss of equilibrium (stage 11) and then slows. At this level the fish are relatively motionless and may rest upright, inverted, or on their sides on the bottom of the container. They can be handled gently, but striking the container or squeezing the caudal peduncle or fin induces strong reflex movements. Bell (1964) indicated that quinaldine was useful for surgical operations on coho salmon, but in operations on rainbow trout we observed periodic reflex movements that hindered surgery.

Fish can be maintained in total loss of equilibrium, (stage 11) for relatively long periods, depending on concentration, before the onset of loss of reflex and medullary collapse. The loss of reflex stage appears to be practically nonexistent. Thus, loss of equilibrium is best suited for evaluating the efficacy of quinaldine.

The mode of action of quinaldine in fish is unknown. Bell (1964) suggested that it may act like the barbiturates as depressants on the central nervous system and especially the respiratory center. We measured opercular rates in anesthetized and control rainbow trout. The rate in the latter was approximately 60 per minute. In treated individuals the rate increased

to more than double the control value after 5 minutes of exposure. Although the rate in treated fish was much faster, the opercular movements appeared weak and were interrupted by periodic gasps.

EFFICACY

Concentrations of 15 or 16 ppm were, in most instances, at least 90 percent effective for inducing anesthesia within 2 minutes in rainbow trout, brown trout, brook trout, and lake trout (table 2). The mean exposures tolerated by the trout ranged from 45 minutes to more than 6 hours. Most individuals recovered in fresh water within several minutes, but some required over 60 minutes. Temperature and size of trout appear to have no influence on efficacy, or on exposure and recovery time.

As many as 25 percent of the test fish died in some trials (table 2). This was not delayed mortality, but resulted from attempts to measure the longest exposure tolerated by each test group. Since the fish could not be observed constantly during the experiments, the most susceptible individuals were overexposed. The procedure may have contributed to the long recovery times noted in some trials. However, the mortalities of trout should be minimal when the progress of anesthesia can be watched more closely.

Channel catfish, bluegills, and largemouth bass are more resistant at temperatures of 17° C. and lower than are salmonids (table 2). Catfish were anesthetized by 70 ppm, bluegill by 15 to 60 ppm, and bass by 20 to 70 ppm. At 22° or 27° C., about 15 ppm were effective on bluegill and bass, and 30 ppm narcotized catfish. The results show that 7- to 12-inch bass are much more resistant to quinaldine than smaller individuals.

The relatively high concentrations required to anesthetize catfish, bluegills, and bass at 17° C. and below shortened the mean exposure and lengthened recovery time (table 2). In general, the fish tolerated exposure for about 5 to 20 minutes at levels exceeding 15 ppm. At lower concentrations they commonly tolerated exposures of one to more than six hours. Recovery time appeared to be more related to temperature and concentration than to exposure time, especially in bluegills and largemouth bass. For example, bluegills recovered in 2 to 4 minutes after a 6-hour exposure to 15 ppm at 27° C., whereas more than 60 minutes were required at 7° after a 0.4-hour exposure to 60 ppm. This may indicate an effect of low temperature on the excretion or metabolic deactivation of quinaldine.

Effects of pH.--The efficacy trials shown in table 2 were carried out at pH 7.0 and 8.5. We combined the data since there appeared to be no difference in the results at these pH values. The trials at pH 5.0 gave quite different results. The drug was completely ineffective on all seven species. Further trials were carried out to determine the approximate degree of acidity which deactivated quinaldine. Nine smallmouth bass were anesthetized in a 20-ppm solution at 12° C. and pH 7.0. The pH was changed to 5.0 and the fish recovered in 20 to 25 minutes. In another experiment the pH was changed to 5.7, but the fish did not recover within a 2-hour period. Thus, a pH of about 6.0 or above does not deactivate quinaldine.

Acidic solutions apparently do not destroy quinaldine. Channel catfish were not affected by a 1-hour exposure to either 50 or 60 ppm of the chemical at pH 5.0. When the pH of the former solution was raised to 7.0, and that of the latter to 10.3, the fish were narcotized in 3 to 10 minutes. This suggests that under acid conditions the quinaldine molecule shifts from an ionic to a non-ionic form which is less biologically active.

Effects of water hardness.--Quinaldine was as effective on rainbow trout in soft water (10 ppm total hardness) as in hard water (180 ppm total hardness). Four trials were run in soft water and two in hard water, and in each case all of the fish were anesthetized within 2 minutes.

Effects of repeated anesthetization.--Daily exposure of rainbow trout to 12 ppm of quinaldine for 6 days did not influence their sensitivity to the drug. The fish were anesthetized within 1 to 3.5 minutes, and variations in efficacy appeared random. They were exposed to the chemical for

Table 2.--Concentrations of quinaldine which anesthetize seven species of fish to total loss of equilibrium (stage II) within 2 minutes

Species	Concentration (ppm)	Temperature (° C.)	Fish: Size (in.)	Fish: Number tested	Fish in anesthesia: (number)	Fish in anesthesia: (percent)	Mean Exposure Time (hrs.)	Safe Exposure Index[1]	Recovery: Mean time range (min.)	Recovery: Survival (percent)
Rainbow trout...	15	7°	2- 6	45	43	96	0.8	24	6-13	100
Do.....	15	7°	7-12	20	19	95	6.0	180	-	95
Do.....	15	12°	7-12	20	19	95	2.3[2]	-	1-4	100
Do.....	15	12°	7-12	20	20	100	6.0	180	-	100
Do.....	15	17°	7-12	25	16	64	6.0	180	3- 5	76
Do.....	16	17°	7-12	25	25	100	3.0	90	5-43	76
Brown trout...	16	7°	7-12	20	20	100	6.0	180	13-23	100
Do.....	16	12°	2- 6	10	10	100	>6.0	>180	-	10
Do.....	16	12°	7-12	15	15	100	6.0	180	5- 8	100
Do.....	16	12°	7-12	5	5	100	4.0	120	<25	100
Do.....	16	17°	7-12	20	20	100	6.0	180	4- 6	100
Brook trout...	16	7°	2- 6	20	20	100	1.7	51	3-21	80
Do.....	16	7°	7-12	20	20	100	6.0	180	15-27	95
Do.....	16	12°	2- 6	40	35	88	1.0	30	3->60	88
Do.....	16	12°	7-12	20	20	100	0.5[2]	-	2- 4	100
Do.....	16	17°	7-12	20	20	100	6.0	180	-	95
Lake trout...	15	7°	2- 6	40	35	88	1.0	30	5-10	75
Do.....	16	7°	2- 6	30	30	100	1.8	54	10->60	90
Do.....	15	7°	7-12	24	24	100	6.0	180	<40	92
Do.....	15	12°	2- 6	100	100	100	2.9	57	2-15	98
Do.....	15	12°	7-12	20	20	100	6.0	180	<30	90
Do.....	15	17°	7-12	24	20	83	6.0	180	6- 9	100
Channel catfish.	70	7°	7-12	42	42	100	0.1	3	11-24	100
Do....	70	12°	2- 6	70	70	100	0.1[2]	-	5-11	100
Do.....	70	12°	7-12	30	30	100	0.1	3	5- 6	100
Do.....	70	17°	2- 6	70	70	100	0.1[2]	-	4- 7	100
Do.....	70	17°	7-12	10	10	100	0.1	3	3- 6	100
Do.....	30	27°	2- 6	10	10	100	1.0	30	3- 4	80
Do.....	30	27°	7-12	5	4	80	6.0	180	<5	100
Do.....	30	27°	7-12	10	10	100	1.2	36	1- 3	100
Do.....	30	27°	7-12	5	5	100	>6.0	>180	1	100
Bluegill..	60	7°	2- 6	37	29	78	0.4	12	>60	92
Do.....	30	12°	2- 6	30	28	93	0.3	9	12->60	100
Do.....	15	17°	2- 6	20	20	100	6.0	180	4- 8	100
Do.....	10	22°	2- 6	20	20	100	0.3	9	20-35	100
Do.....	15	27°	2- 6	30	30	100	1.0	30	2-20	93
Do.....	15	27°	2- 6	10	10	100	6.0	180	2- 4	100
Largemouth bass....	30	7°	2- 6	20	19	95	1.8	54	36->60	95
Do.....	70	7°	7-12	5	5	100	0.1	3	40->60	100
Do.....	15	12°	2- 6	60	59	98	1.8	54	10-33	92
Do.....	30	12°	7-12	70	70	100	0.1[2]	-	8-13	100
Do.....	20	17°	7-12	21	19	90	0.4[2]	-	6-29	95
Do.....	16	22°	2- 6	20	20	100	2.5	75	2- 3	95
Do.....	15	27°	2- 6	40	39	98	4.5	135	1- 2	100

1/ Index obtained by dividing the time for the first fish to reach medullary collapse by the time (2 minutes) for fish to reach total loss of equilibrium, stage II.

2/ Fish removed from the anesthetic before reaching medullary collapse.

periods of approximately 2 to 20 minutes, and all recovered in fresh water within 2 minutes. The response of fish receiving six treatments was essentially the same as those not treated previously.

Effects of aging.--The aging of quinaldine solutions at 12° C. appears to have little effect on efficacy. Concentrations of 15 ppm which were aged for 50 days anesthetized rainbow trout within 2.0 minutes. This compares favorably with the effectiveness of solutions bioassayed immediately after preparation. At 27° C. quinaldine was less effective on bluegill after the solutions were aged for 21 days (table 3). These data show that solutions of the anesthetic should be usable over relatively long periods, unless fouled by mucous or excrement, but they may require aeration. We found that aeration for as long as 24 hours does not diminish efficacy.

Table 3.--Influence of aging on efficacy of 12 ppm of quinaldine for bluegills at 27° C.

Solution age (days)	Number of fish	Fish in loss of equilibrium stage 11		Recovery in fresh water (number)
		Number	Time (Min.)	
0	10	10	1.0-2.0	10
1	10	10	0.5-1.5	10
3	10	10	1.0-2.0	10
7	10	10	0.5-1.0	10
14	10	10	0.5-2.5	10
21	10	0	-	10
21	10	4	7.0-9.0	10

Repeated use of solutions.--The approximate "life expectancy" of quinaldine solutions was determined by anesthetizing groups of rainbow trout in a concentration of 15 ppm. A total of 3,542 grams of fish were treated in a solution containing 0.037 ml. of quinaldine before the exposure required for anesthesia exceeded 2 minutes. This ratio of fish weight to quinaldine amounts to 94.5 kg./ml. Meister and Ritzi (1958) made similar measurements for MS-222. They found that about 14 kg. of brook trout and 42 kg. of lake trout could be effectively narcotized per gram of drug.

TOXICITY TO EGGS

Fifteen- to 30-minute exposures of fertilized rainbow trout eggs to concentrations as high as 120 ppm of quinaldine were apparently not detrimental (table 4). Two-hour exposures to concentrations of 60 and 120 ppm killed approximately 40 and 100 percent of the eggs respectively within 4 days, while mortalities at 15 and 30 ppm were relatively light. Some random mortalities occurred in the treated and control eggs throughout the balance of the incubation period. The remaining eggs hatched approximately 21 days later. Since most spawning operations would probably be carried out with concentrations of 15 to 30 ppm, and since fertilized eggs are placed subsequently in flowing water, it is unlikely that accidental contamination of eggs would be detrimental.

Table 4.--The toxicity of quinaldine to fertilized, rainbow trout eggs at 12° C.

Concentration (ppm)	Number of eggs	Number of dead eggs within 4 days after exposure to quinaldine for			
		0.25 hours	0.50 hours	1.00 hours	2.00 hours
Control	60	0	0	0	0
15	15	0	0	0	1
30	15	0	0	0	1
60	15	0	0	2	6
120	15	0	0	1	15

METABOLISM OF QUINALDINE

The metabolism of quinaldine in fish has not been elucidated. Early investigations with other animals indicated that the chemical may be converted into natural metabolites. Kusui (1931) concluded that frogs oxidized a small portion of subcutaneously injected quinaldine to quinaldic acid which was excreted in the urine. Takahashi (1931) reported that chickens and rabbits transformed the compound into alpha-quinolinic acid, some of which was conjugated with glycine. Later studies have shown that relatively large amounts of 4-methylquinoline

(lepidin) are oxidized by chickens, but only trace quantities of 2-methylquinoline (quinaldine) are changed to quinaldic acid (Tsunoo et al., 1965). Quinaldic acid is a normal metabolite of tryptophan in various mammals and may be conjugated with glycine and excreted in the urine (Roy and Price, 1959; Kaihara, 1960; Kaihara and Price, 1961). Thus, even in homotherms, the metabolic fate of quinaldine is not well understood.

SUMMARY

Concentrations of 15 or 16 ppm of quinaldine rapidly induce total loss of equilibrium in trout. Fifteen to 30 ppm are effective on channel catfish, bluegills, and largemouth bass at temperatures of 22° and 27° C. At lower temperatures, concentrations up to 60 or 70 ppm are needed to achieve rapid anesthesia in the largemouth bass. Quinaldine solutions with a pH of 5.0 failed to anesthetize fish, but efficacy was restored by increasing basicity. Water hardness, age of solutions, and repeated exposures of fish to quinaldine appear to have little influence on efficacy. The chemical is toxic to fertilized rainbow trout eggs only after several hours exposure to relatively high concentrations.

The utility of quinaldine as a fish anesthetic depends in large part on the needs of the fishery worker. Its major assets include rapid action and prolonged maintenance of anesthesia. On the other hand, anesthetized fish retain a degree of reflex responsiveness which may interfere with stripping, delicate surgical operations, and blood collection.

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INVESTIGATIONS IN FISH CONTROL

23. Toxicity of Quinaldine to Selected Fishes

By Leif L. Marking, Chemist

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
Washington, D.C. · January 1969

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TOXICITY OF QUINALDINE TO SELECTED FISHES

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ABSTRACT.--Quinaldine, an anesthetic for fish, is toxic to various sizes of rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegills, largemouth bass, and walleyes in 15-, 30-, and 60-minute and 3-, 6-, 24-, 48-, and 96-hour static bioassays. Toxic concentrations range from 2.0 to 25 ppm in standard tests at 12° C. in 96 hours. Its toxicity to rainbow trout is significantly greater at higher temperatures, and 96-hour LC50's range from 13.3 ppm at 7° to 1.9 ppm at 17° C. In 6-hour exposures, quinaldine is more toxic at colder temperatures. Bluegills are more sensitive at 22° than at 12° or 17° C. The anesthetic is more toxic to fish in hard than in soft water, a condition probably associated with pH. Safety indexes show that shorter exposures to quinaldine are safer to fish, although the concentrations may be greater than required in longer exposures. Recovery from anesthesia is good among survivors in fish exposed to partial-kill concentrations of quinaldine for 96 hours.

Anesthetics are rapidly becoming more important and widely used in fisheries. Varieties of anesthetics have been found useful in marking, tagging, transporting, and spawning fish of various species (Parkhurst and Smith, 1957; McFarland, 1960; Bell, 1964). Practical concentrations of chemicals to produce desirable anesthesia in fish have been defined under field conditions by several workers (Meister and Ritzi, 1958; Thompson, 1959). Klontz (1964) outlined 14 methods used to anesthetize fish.

Quinaldine was reported to be an anesthetic for fish by Muench (1958). More recently, Matarajan and Ranganathan (1960) and Greenough (1963) discussed the usefulness of quinaldine in fisheries. A comprehensive study of the compound's efficacy as an anesthetic for seven freshwater fish species was made by Schoettger and Julin (1968).

Quinaldine (2-methylquinoline) occurs in coal tar. It can be manufactured by treatment of aniline and paraldehyde with hydrochloric acid and heat (Rose and Rose, 1966) or derived from aniline, acetaldehyde, and hydrochloric acid (Stecher et al., 1960). Quinaldine boils at 246°-247° C., darkens from light yellow to brown with exposure to air, is soluble in alcohol, ether, chloroform, and acetone, but is insoluble in water (Rose and Rose, 1966).

This study was undertaken to establish the toxicity of practical-grade quinaldine to nine species of fish.

METHODS AND MATERIALS

Nine species of fish were obtained from fish hatcheries (table 1). Three size groups included 1- to 3-inch, 3- to 5-inch, and 6- to 9-inch fish.

Table 1.--Fishes used in tests of quinaldine

Common name	Scientific name	Source
Rainbow trout....	Salmo gairdneri	NFH, Manchester, Iowa
Brown trout.....	Salmo trutta	NFH, Manchester, Iowa
Brook trout.....	Salvelinus fontinalis	SFH, Osceola, Wis.
Lake trout.....	Salvelinus namaycush	NFH, Jordan River, Mich.
		SFH, St. Croix Falls, Wis.
Northern pike....	Esox lucius	NFH, Garrison Dam, N. D.
		NFH, Gavins Point, S. D.
Channel catfish..	Ictalurus punctatus	NFH, Fairport, Iowa
Bluegill.....	Lepomis macrochirus	NFH, Lake Mills, Wis.
Largemouth bass..	Micropterus salmoides	NFH, Genoa, Wis.
Walleye.....	Stizostedion vitreum	NFH, Garrison Dam, N. D.

Ten fish were included at each of 10 or 11 concentrations of quinaldine in 15-liter, static bioassays as described by Lennon and Walker (1964). Ten to twenty of the 1- to 3-inch fish served as controls depending on how many concentrations were tested. The bioassays with 3- to 5-inch and 6- to 9-inch fish were made in polyethylene tanks containing 45 liters of aerated solution. Five concentrations were tested against the 9-inch fish, and 10 fish served as controls.

Variations in water quality were arranged by adding different amounts of reconstituting salts to deionized water (table 2). Temperatures of 7°, 12°, 17°, and 22° C. were maintained by placing the bioassay vessels in thermostatically controlled water baths. All temperatures listed hereafter are in Centigrade.

Concentrated stock solutions of the practical-grade quinaldine manufactured by Eastman Organic Chemicals were mixed daily to insure complete activity and prevent degradation. Acetone was used to dissolve 7.5 milliliter of quinaldine and this was diluted with deionized water. Approximately 50 percent of the final 250 milliliter of stock volume was water.

Fish responses to quinaldine were recorded for several hours after exposure and daily thereafter throughout the 96-hour bioassay. Dead fish were recorded and removed. Live fish were readily distinguishable because they were hypersensitive to sound and vibrations up to the time of death. Fish which remained in anesthesia throughout a test were placed in fresh water until they recovered. The times for recovery and survival were noted.

The toxicity data were analyzed according to the methods of Litchfield and Wilcoxon (1949) to determine LC50's, variations, slope functions, and 95 percent confidence intervals.

Safety indexes were calculated to determine the margin of safety between efficacious and lethal concentrations of quinaldine. The values

Table 2.--Quantities of salts added to deionized water at the Fish Control Laboratories

Classification of water	Salt added in mg./l.				pH range	Concentration as ppm $CaCO_3$	
	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCL		Total hardness	Total Alkalinity
Soft.....	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Standard 1/	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Medium....	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Hard.....	384	240.0	240.0	16.0	8.0-8.4	280-320	225-245

1/ Standard reconstituted water used in routine bioassay.

derived are the quotients of effective and lethal concentrations.

RESULTS

SPECIES AND SIZES OF FISH

Quinaldine is toxic to coldwater and warmwater fish in 96-hour exposures, and LC50's range from 2.0 to 24.9 ppm (table 3). Channel catfish are the most resistant species irrespective of size and duration of exposure. Northern pike show a decreased resistance between LC50's of 20 ppm at 24 hours and 2 ppm at 96 hours. The decrease may be attributed to a combination of starvation and quinaldine intoxication. This species demands a large and constant supply of food, but none is supplied in the bioassay.

The small sizes of rainbow trout, brown trout, lake trout, channel catfish, and largemouth bass are more sensitive to quinaldine than large individuals, particularly at 96-hour exposures (table 3). The LC50 for 2-inch rainbow trout, for example, is 5.0 ppm while the LC50 for 6-inch fish is 15.3 ppm; and the LC50's for 2- and 6-inch brown trout are 3.5 and 14.0 ppm, respectively.

The 2-inch rainbow trout are more resistant at 24 and 48 hours in exposures to quinaldine, than at 96 hours. Two-inch brown trout respond similarly. The LC50's for lake trout, on the other hand, do no vary significantly between the 48- and 96-hour exposures.

The toxicity of quinaldine was relatively uniform to brook trout of the sizes tested. Larger individuals appeared to be more sensitive, but also had become infected with furunculosis just prior to the bioassays. The added stress factor may explain these results. The disease in these fish was diagnosed following the tests. In general, brook trout resistance to the anesthetic was similar to that of the larger rainbow and brown trout.

EFFECTS OF TEMPERATURE

In 1- to 6-hour tests at 7°, 12°, and 17°, rainbow trout are more resistant to the toxic effects of quinaldine at 17° than at 12° or 7° (table 4). This relationship is reversed at 24 hours, and rainbow trout are more sensitive at 17°. The 1- and 96-hour LC50's range from 17.8 to 13.3 ppm at 7° and from 23.8 to 1.9 ppm at 17°. The 96-hour LC50's show significant differences in toxicity.

Rainbow trout temperature tests were repeated several times since survival and mortality were erratic and results were difficult to analyze statistically. The 12° test in table 4, for instance, indicates 50-percent mortality at 5 ppm. Concentrations of 6 to 12 killed all test animals while one of ten survived 14, 18, and 20 ppm at 96 hours. These data indicate that quinaldine is inconsistent in its toxic effects at high concentrations.

Higher temperatures of 12°, 17°, and 22° were used to determined the effects of quinaldine on bluegills (table 5). The LC50 of 10.1 ppm for 24, 48, and 96 hours indicates that exposures over 24 hours do not increase the toxicity. The effect of exposure was also small at 17° but bluegills are more resistant than at 12° or 22°. The toxicity of quinaldine at 22° increases significantly at 96 hours exposure and bluegills die at approximately 6 ppm.

EFFECTS OF WATER QUALITY

Quinaldine toxicity to 2-inch rainbow trout is essentially the same in standard and medium quality water after 1, 3, 6, and 96 hours exposure (table 6). Quinaldine is significantly less toxic in soft water, however, in 1- to 6-hour exposures. It is also less toxic in soft water at 96 hours, but the difference in soft, standard, and medium water quality is not significant at this time interval. Toxicity increases with exposure time at every water quality.

Rainbow trout survival in soft and medium quality water was erratic and some fish lived in surprisingly high concentrations of drug. In water of medium hardness one trout survived 20 ppm of quinaldine, but none survived concentrations between 6 and 20 ppm. In soft water one trout survived 18 ppm of the anesthetic while none survived concentrations between 8 and 18 ppm. Survival occurred at the higher concentrations,

Table 3.--Toxicity of quinaldine to fish at 12° C.

Species	Average weight (grams)	Approximate length (inches)	LC50 (ppm) and 95 percent confidence interval at 24 hours	48 hours	96 hours
Rainbow trout....	1.0	2	18.7 18.2-19.2	17.8 16.4-19.3	5.0 4.5-5.6
Do.....	23.0	6	16.0 14.2-18.1	15.3 13.9-16.8	15.3 13.9-16.8
Brown trout.....	2.6	2	13.0 10.9-15.5	9.0 6.0-13.5	3.5 2.1-5.9
Do.....	14.3	4	18.0 15.3-21.2	17.0 14.9-19.4	16.0 14.7-17.4
Do.....	27.0	6	15.0 13.6-16.5	14.8 13.5-16.3	14.0 12.7-15.4
Brook trout.....	12.5	3	14.5 13.1-16.1	14.0 12.8-15.3	13.6 12.5-14.8
Do.....	20.0	4	15.0 13.2-17.1	14.0 13.1-15.0	13.5 12.6-14.4
Do.....	37.5	6	13.2 12.5-14.0	12.4 11.4-13.5	12.0 10.7-13.4
Lake trout.....	2.0	2	6.8 5.8-8.0	5.6 5.0-6.3	5.6 5.0-6.3
Do.....	5.6	3	14.2 13.4-15.1	13.5 12.4-14.7	13.5 12.4-14.7
Do.....	35.0	7	13.0 12.1-13.9	12.6 11.7-13.6	12.3 11.3-13.4
Northern pike....	1.8	2	20.0 18.8-21.2	8.0 6.3-10.2	2.0 1.1-4.6
Channel catfish..	1.9	3	21.0 19.3-22.9	20.0 18.2-22.0	19.9 18.1-21.9
Do.....	5.2	4	29.4 28.3-30.6	27.4 25.6-29.3	24.9 23.3-26.6
Bluegill.....	1.3	2	10.1 9.4-10.8	10.1 9.4-10.8	10.1 9.4-10.8
Do.....	2.8	3	12.8 12.2-13.4	12.8 12.2-13.4	12.6 11.8-13.5
Largemouth bass..	0.5	1	10.4 9.7-11.1	9.4 8.5-10.3	4.6 3.5-6.1
Do.....	5.2	4	10.4 9.8-11.0	9.9 9.3-10.5	6.5 5.6-7.5

(continued) Table 3.--Toxicity of quinaldine to fish at 12° C.

Species	Average weight (grams)	Approximate length (inches)	LC50 (ppm) and 95 percent confidence interval at 24 hours	48 hours	96 hours
Largemouth bass..	63.0	7	10.0 8.8-11.3	9.7 8.7-10.9	9.0 7.7-10.5
Walleye	0.7	2	10.1 9.4-10.9	10.1 9.3-11.0	9.8 8.9-10.8

Table 4.--Toxicity of quinaldine to rainbow trout at three temperatures

Temperature ° C.	LC50 (ppm) and 95 percent confidence interval at 1 hour	3 hours	6 hours	24 hours	48 hours	96 hours
7°.....	17.8 16.2-19.4	16.1 15.6-16.7	16.1 15.6-16.7	15.5 14.3-16.8	14.2 12.6-15.9	13.3 11.9-14.9
12°.....	19.8 18.8-20.8	19.8 18.8-20.8	19.8 18.8-20.8	18.7 18.2-19.2	17.8 16.4-19.3	5.0 4.5-5.6
17°.....	23.8 21.5-25.4	23.8 21.5-25.4	23.0 20.3-26.0	8.0 6.2-10.1	3.2 2.3-4.5	1.9 1.5-2.3

Table 5.--Toxicity of quinaldine to bluegills at three temperatures

Temperature ° C.	LC50 (ppm) and 95 percent confidence interval at 24 hours	48 hours	96 hours
12°....	10.1 9.4-10.8	10.1 9.4-10.8	10.1 9.4-10.8
17°....	12.5 11.9-13.1	11.8 10.9-12.7	11.6 10.7-12.5
22°....	11.3 10.6-12.1	11.0 10.0-12.1	5.8 5.6-6.0

but mortality was not erratic or unusual at the lower end of the lethal range. The trials in various water qualities were repeated several times to confirm the variations in survival.

RECOVERY

Fish exposed to sublethal concentrations of quinaldine usually recovered from anesthesia in the test vessel within the 96-hour bioassay. Anesthesia progresses to partial or total loss of equilibrium within 15 to 30 minutes. The effects remain for 3 to 6 hours and then diminish. Intermediate concentrations produced anesthesia much faster and killed fish at progressively higher concentrations. Fish surviving the partial kill range, but still in deep anesthesia at 96 hours, were removed to fresh water and recovery was noted. Recovery was considered complete when the fish could swim against a current.

Table 6.--Toxicity of quinaldine to rainbow trout in selected water qualities at 12° C.

Water quality[1/]	LC50 (ppm) and 95 percent confidence interval at					
	1 hour	3 hours	6 hours	24 hours	48 hours	96 hours
Soft.....	25.0 21.8-28.7	25.0 21.8-28.7	24.1 20.8-27.8	19.0 17.1-21.2	17.0 15.3-18.8	5.1 4.4-6.0
Standard.	19.8 18.8-20.8	19.8 18.8-20.8	19.8 18.8-20.8	18.7 18.2-19.2	17.8 16.4-19.3	5.0 4.5-5.6
Medium...	19.1 17.4-21.0	18.5 16.9-20.3	18.4 16.9-20.1	16.7 15.2-18.3	14.5 12.6-16.7	4.6 4.2-5.1
Hard.....	21.1 18.5-24.0	20.9 18.4-23.8	20.9 18.4-23.8	17.2 13.8-21.5	9.9 6.8-14.4	4.3 3.7-5.0

[1/] Water qualities correspond to table 2.

Northern pike and channel catfish which survive partial kill concentrations appear to recover faster than the other species and without additional mortality (table 7). The partial kill concentrations for these species are relatively high. Considering all species, the recovery is approximately 95 percent after 96-hour exposures, and all except bluegills recover quite rapidly. Occasionally one or several of a group will require longer periods. This fact was noted especially among lake trout and largemouth bass.

SAFETY INDEXES

The safety indexes for rainbow trout indicate greater safety in 15-minute exposures than in 30- or 60-minute exposures (table 8). The EC50 refers to the concentration of quinaldine producing total loss of equilibrium in one-half of the specimens. This stage and other stages of anesthesia are described by Schoettger and Julin (1967).

The maximum safety index is based on concentrations producing 99-percent effective anesthesia (EC99) and 1-percent mortality (LC1). These values were extrapolated from the regressions used in determining the EC50 and LC50. The maximum safety index is lower than the safety index and is biased in favor of greater safety. Maximum safety indexes also indicate greater safety in 15-minute exposures (table 8). Indexes for 30- and 60-minute exposures are 0.9 and 1.0 and appear marginal for practical applications of quinaldine. Values less than 1.0 indicate that one must expect mortality if 99 percent of the fish are anesthetized.

DISCUSSION

When the toxicities of quinaldine and MS-222, another fish anesthetic are compared, it is apparent that both drugs elicit similar patterns of toxic response among various species and sizes of fish. Previous research indicates that channel catfish are more resistant than other species to quinaldine and MS-222 (Marking, 1967, and Schoettger et al. 1967). Smaller sizes of fish are generally more sensitive to the anesthetics, and rainbow trout respond erratically in waters of various qualities. Among the trout, lake trout are more sensitive to both anesthetics. The drug safety indices indicate that shorter exposures are safer and minimize mortality.

Actually, quinaldine is more toxic to fish than MS-222. The LC50's for quinaldine range from 2.0 to 24.9 ppm while those for MS-222 range from 32.0 to 62.1 ppm for all species tested in 96-hour exposures at 12°. However, quinaldine is effective as an anesthetic at lower concentrations than MS-222 so that the safety indexes for both are quite similar.

In contrast with MS-222 quinaldine is more toxic at colder temperatures to 2-inch rainbow trout in 1- to 6-hour exposures. Lethal intoxication is not apparent in brief exposures at warmer temperatures, but toxic effects are

Table 7.--Recovery from anesthesia of fish exposed to concentrations of quinaldine causing partial kills within 96 hours at 12° C.

Species (all sizes)	Partial kill concentration (ppm)	Number of fish		Minutes to recover in fresh water
		Surviving at 96 hours	Recovering after 96 hours	
Rainbow trout ..	5-16	105	102	2-60
Brown trout.....	1-16	53	49	4-90
Brook trout.....	10-16	43	42	3-60
Lake trout.....	6-14	24	21	4-120
Northern pike...	10-24	24	24	5-35
Channel catfish.	12-36	97	97	4-16
Bluegill.....	9-14	73	71	19-75
Largemouth bass.	2-11	68	60	5-120
Walleye.....	3-10	38	35	5-60

Table 8.--Safety and maximum safety indexes of quinaldine against 2-inch rainbow trout in brief exposures at 12° C.

Exposure (minutes)	Safety index			Maximum safety index		
	LC50 (ppm)	EC50 (ppm)	LC50/EC50	LC1 (ppm)	EC99 (ppm)	LC1/EC99
15....	35.2	13.6	2.6	25.0	20.0	1.3
30....	25.2	14.0	1.8	18.5	20.0	0.9
60....	22.4	13.3	1.7	17.0	16.4	1.0

manifest after 24 hours. Quinaldine is also more toxic to bluegills at higher temperatures but only after 48 hours of exposure.

Quinaldine is less toxic to 2-inch rainbow trout in soft than in harder waters. Marking (1967) found little difference in the toxicity of MS-222 to rainbow trout at various water hardnesses. The low salt content and correspondingly low pH value of the soft water apparently affect the activity of quinaldine. Schoettger and Julin (1968) observed the effects of pH on quinaldine and noted that the drug is completely ineffective on fish at pH 5°. These data agree with the decreased toxicity of quinaldine in soft water.

CONCLUSIONS

Quinaldine is toxic to fish, and the 96-hour LC50's for nine species range from 2.0 to 24.9 ppm. Channel catfish are the most resistant to the drug.

The anesthetic is more toxic to small fish, especially at 96 hours exposure. It is also more toxic to small rainbow trout and bluegills at higher temperatures in the longer exposure period.

Small rainbow trout respond erratically to the toxicity of quinaldine at various temperatures and water qualities. The fish are more resistant to the drug at higher temperatures in 1- to 6-hour exposures but less resistant in 24- to 96-hour exposures. They are also more resistant to the anesthetic in soft water than in harder waters.

The safety indexes for quinaldine on fish indicate that brief exposures are safer for the fish and minimize mortalities.

Recovery from anesthesia is good among survivors exposed to partial-kill concentrations of quinaldine for 96 hours. The recoveries of nine species in fresh water occur within 2 to 120 minutes. The process of recovery from anesthesia in static test solutions begins during the bioassay after 6 hours of exposure.

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INVESTIGATIONS IN FISH CONTROL

24. Quinaldine as an Anesthetic for Brook Trout, Lake Trout, and Atlantic Salmon

By David O. Locke

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
Washington, D.C. · January 1969

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QUINALDINE AS AN ANESTHETIC FOR BROOK TROUT, LAKE TROUT, AND ATLANTIC SALMON

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ABSTRACT.--Quinaldine (2-methylquinoline) was an effective anesthetic for yearling Atlantic and landlocked salmon and brook and lake trout in waters ranging from 10 to 40 ppm total hardness and temperatures ranging from 36° to 40° F. and from 47° to 59° F. Lake trout were more sensitive than the other species tested. In tests, anesthetization and recovery rates for five concentrations (5, 10, 15, 20, and 25 ppm) at both temperatures (10 ppm) was generally satisfactory for lake trout. A concentration of 15 ppm was satisfactory for marking and general handling of salmon and brook trout. Quinaldine is one twenty-fourth as expensive as MS-222 at 1:12,000, and in view of our excellent results this drug warrants wider use as a fish anesthetic.

The comparatively high cost of the popular MS-222 as an anesthetic for fish has prompted many fishery workers to consider less costly drugs as substitutes. This paper reports on tests of quinaldine for anesthetizing several coldwater fishes. Quinaldine (2-methylquinoline) is currently available in practical grade from Distillation Products Industries, Division of Eastman Kodak Company, at $14.35 per 500-gram bottle. Quinaldine is also used in the manufacture of dyes and explosives. It has not been used in medicine as have other quinolines, but according to Muench (1958) it may have some antiseptic value. The exact mode of action of quinaldine on fish is unknown, but it supposedly acts like barbiturates, depressing the central nervous system, especially the respiratory center.

Although quinaldine is used extensively by fishery workers as an anesthetic, little has been published on its use. Muench (1958) reported its use on green sunfish, white crappie, yellow bullhead, golden shiner, and goldfish. Leitritz (1962) mentioned that the concentrations used range from 5 to 12 ppm. Bell (1964) recommended doses of 6.6 to 10 ppm for 10-inch coho salmon. We decided to determine the usefulness of quinaldine for anesthetizing the commonly handled salmonids in the soft waters found in Maine.

I wish to thank Donald F. Mairs for his assistance and advice. Dr. W. Harry Everhart, Robert E. Foye, and Robert S. Rupp critically reviewed the manuscript and made many helpful suggestions.

MATERIALS AND METHODS

A stock solution was prepared by mixing 37.85 milliliter of quinaldine with 40 milliliter of acetone and enough distilled water to make 1 liter. The stock solution maintains its effectiveness for long periods when stored in brown bottles. One milliliter of stock solution added to 1 gallon of water gives a concentration of 10 ppm.

Tests were conducted on yearling Atlantic and landlocked salmon (Salmo salar), brook trout (Salvelinus fontinalis), and lake trout (S. namaycush) in waters ranging from 10 to 40 ppm total hardness and at temperatures ranging from 36° to 40° F. and from 47° to 59° F. Five concentrations were tested: 5, 10, 15, 20, and 25 ppm. Each test was performed in duplicate. Control fish were handled exactly the same as test fish except that they were placed in containers of untreated fresh water. Each test consisted of placing six fish in a Fernow pail containing 5 gallons of solution. We recorded the time required for fish to recover from anesthesia by

placing them in wash tubs containing 5 gallons of fresh water. All fish, including the controls, were fin-clipped for identification and subsequently held in raceways for 2 weeks to observe delayed mortality.

Each fish was considered anesthetized when it remained quietly on the bottom of the pail and exhibited no movement other than respiration and an occasional flexure of the caudal fin. Recovery was considered complete when the fish righted itself and maintained its equilibrium. Anethetization time was recorded for the first, third, and last fish. Each treatment was terminated after the fish had been in the test solution for 15 minutes. All fish were then placed in fresh water, and recovery time of the first, third, and last fish was recorded.

RESULTS

Anesthetization and recovery rates of different kinds and sizes of fish were directly proportional to the concentration of quinaldine in both temperature ranges (figs. 1-4). Generally the fish were anesthetized quicker at the higher temperature and also recovered sooner than at the lower temperature. The greatest contrast in rate of anesthetization relative to concentration occurs at the lower temperatures. It takes 5 to 7 times as long to anethetize brook trout at 5 ppm than at 10 or 15 ppm at 36° F. Although the efficacy was not affected to this degree on the other species, the 5 ppm concentration did not provide dependable and speedy anethetization for these ranges in temperature.

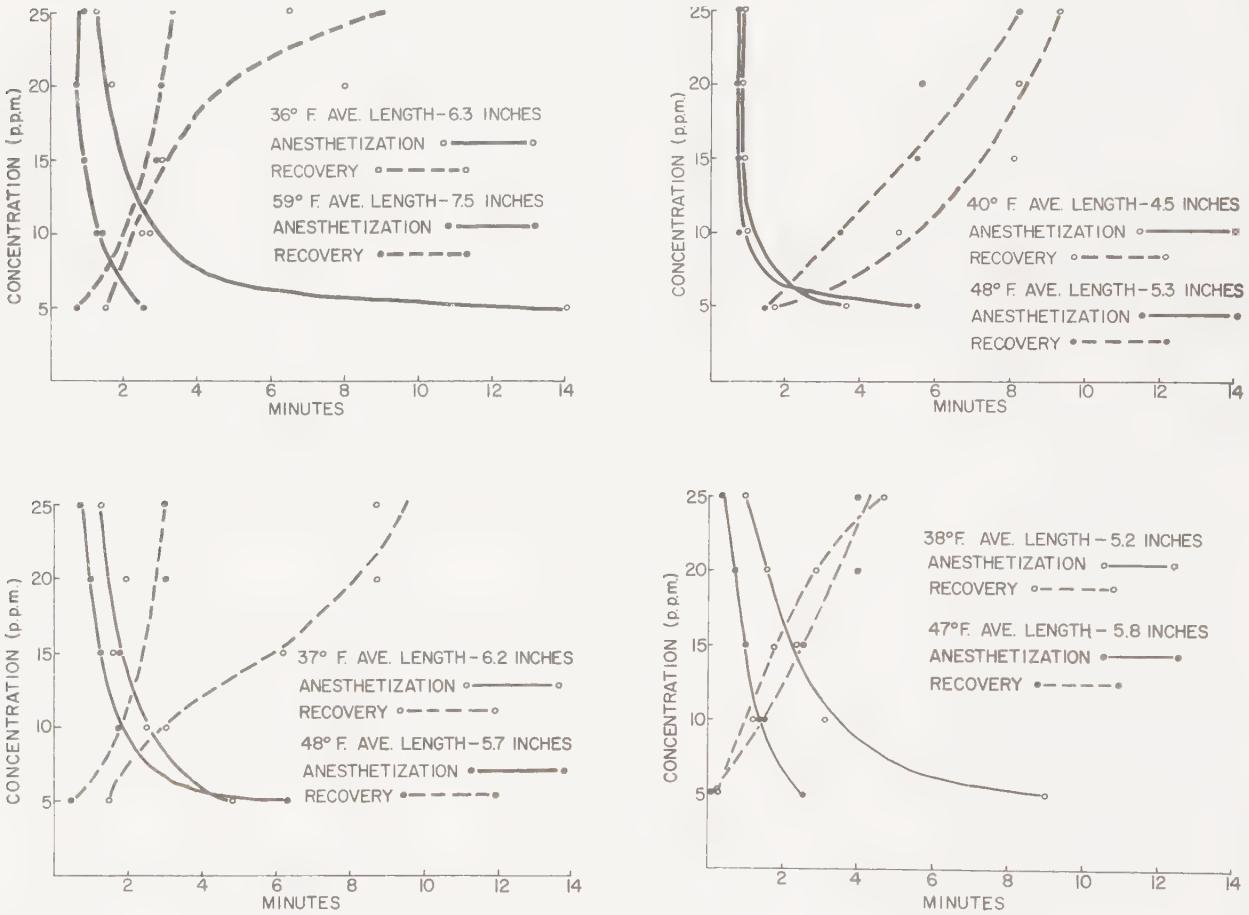

Figure 1.--Effect of quinaldine on brook trout (top left), lake trout (top right), Atlantic salmon (bottom left), and landlocked salmon (bottom right) at two temperature levels. Reported times are averages of two trials and indicate the anesthetization and recovery of one-half of the test fish.

The muscles of anesthetized fish became relaxed and the fish did not respond to gentle handling. Rough handling and sudden disturbances sometimes caused the anesthetized fish to swim a short distance and then come to rest again. Larger fish appeared to be more deeply narcotized than smaller ones.

The respiration rate of effectively anesthetized fish appeared to be more rapid than that of the control fish. Opercular movements were slight. Respiratory movements did not cease among any of the species at any of the concentrations tested.

Anesthetized fish retained their dark coloration, while the control fish became very light in response to the light background of the test container. This failure to change color is probably the result of the anesthetic upon the central nervous system.

Recovery began with a shivering movement that gradually increased in intensity until the fish had recovered fully. Lake trout gasped at the surface during recovery.

Generally, the anesthetization rates at the higher temperatures were only slightly greater than those in the lower temperatures. Recovery rates were notably higher in warmer water, presumably because of the increased rate of metabolism. The recovery rates in both temperature ranges were well within acceptable limits for all concentrations tested.

Muench (1958) reported that fish exposed to effective concentrations of quinaldine for as long as 2 and 3 days recovered within a few minutes when transferred to fresh water. He also stated that green sunfish held for 11 hours in a concentration three times greater than that necessary for anesthesia suffered no ill effects. In our experiments, all fish were marked and held for 2 weeks for observation of delayed mortality. None was observed.

CONCLUSIONS

Lake trout were more sensitive to anesthesia with quinaldine than were the other species tested. Under most conditions, a concentration of 10 ppm is satisfactory for lake trout. Salmon and brook trout are more tolerant to quinaldine than lake trout, so a concentration of 15 ppm is suggested for these species.

Quinaldine at 15 ppm is 24 times cheaper than MS-222 at 1:12,000. In view of our excellent results and the difference in cost, this drug warrants wider use as a fish anesthetic.

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